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ORIGINAL ARTICLE

# A randomized double-blind, placebo-controlled clinical phase IIa trial on safety, immunomodulatory effects and pharmacokinetics of EA-230 during experimental human endotoxaemia

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**Aims:** EA-230 is a human chorionic gonadotropin hormone-derived linear tetrapeptide, developed for the treatment of systemic inflammation-related disorders. EA-230 has shown promising immunomodulatory and tissue-protective effects in animals and an excellent safety profile in human phase I studies that we performed. The present phase IIa study follows-up on these results by investigating the safety, efficacy and pharmacokinetics of EA-230 under systemic inflammatory conditions induced by experimental human endotoxaemia.

**Methods:** In this randomized, double blind, placebo-controlled phase IIa study, systemic inflammation was induced by intravenous administration of *Escherichia coli*-derived lipopolysaccharide (LPS). At $t$ = 0 hours, 36 healthy male volunteers received 2 ng/kg LPS, followed by a 2-hour continuous infusion of EA-230 (15, 45 and 90 mg/kg/h, $n$ = 8 per group) or placebo ($n$ = 12).

**Results:** EA-230 was well tolerated and showed a favourable safety profile. Treatment with the highest dose of EA-230 resulted in a significant attenuation of the LPS-induced increase in plasma levels of inflammatory mediators interleukin (IL)-6, IL-8, IL-1 receptor antagonist, monocyte chemoattractant protein-1, macrophage inflammatory proteins-1α and -1β, and vascular cell adhesion protein-1 (% reduction of 48, 28, 33, 28, 14, 16 and 19 respectively, $p < .01$), and reduced fever (peak decrease from 1.8 ± 0.1°C to 1.3 ± 0.2°C, $P < .05$) and symptom scores (peak decrease from 7.4 ± 1.0 to 4.0 ± 1.2 points, $P < .05$). EA-230 exhibited a very short elimination half-life and a large volume of distribution in the highest dosage group (geometric mean and 95% confidence interval: 0.17 [0.12–0.24] hours and 2.2 [1.3–3.8] L/kg, respectively).

**Conclusion:** Administration of EA-230 is safe and results in attenuation of the systemic inflammatory response in humans.

Trial registration: www.clinicaltrial.gov, NCT02629874

The authors confirm that the PI for this paper is Peter Pickkers and that he had direct clinical responsibility for the study participants.

KEYWORDS

cytokines, drug safety, immunotherapy, inflammation, pharmacokinetics

## 1 | INTRODUCTION

Systemic inflammation can arise due to a variety of infectious as well as non-infectious conditions such as sepsis, trauma and major surgical interventions.[1,2] Although the immune response is essential for clearance of pathogens and initiation of tissue repair, a too persistent or overzealous response is detrimental and can lead to pronounced tissue damage with associated organ failure and mortality rates up to 30%.[3,4] This is for instance exemplified by the high incidence of acute kidney injury (AKI) in systemic inflammation-related conditions, occurring in over a half of critically ill patients and in up to 30% of patients undergoing elective cardiac surgery.[5-8]

Despite its tremendous impact, no immunomodulatory interventions have proven to be effective in regulating the systemic inflammatory response to prevent organ injury.[9-11] Current strategies are confined to supportive treatment and novel strategies aimed at attenuating this exaggerated inflammatory response to prevent organ failure are therefore highly warranted.

EA-230 is a synthetic linear tetrapeptide (alanine–glutamine–glycine–valine; AQGV) derived from the β-chain of the human chorionic gonadotropin hormone (β-HCG). The discovery of this peptide finds its roots in the unique immunological situation encountered during pregnancy, in which the maternal immune system is adapted to tolerate the semi-allogeneic fetus without compromising pathogen clearing capacity.[12,13] A striking hallmark of this immune-tolerant phenotype is attenuated disease activity of various autoimmune diseases such as rheumatoid arthritis, multiple sclerosis and psoriasis during pregnancy and their relapse after delivery.[14-17] HCG was shown to play a central role in this immune-tolerant adaption of the maternal immune system,[18-21] which has led to the discovery of immune active fragments of the β-chain. These oligopeptides were found to circulate abundantly throughout pregnancy and to exert beneficial effects in various animals models of systemic inflammation, including attenuation of the inflammatory response, less organ failure and improved survival.[22-31] Of these peptides, the linear tetrapeptide AQGV (later named EA-230) strongly attenuated inflammation, preserved kidney function, and substantially reduced mortality in models of renal ischaemia–reperfusion,[24] kidney transplantation,[25] and haemorrhagic-[28] and endotoxaemia-induced shock.[26]

Following these encouraging preclinical results, we performed phase I studies with different dosing regimens that showed EA-230 to be well-tolerated with an excellent safety profile (reported elsewhere in this issue[32]).

The present phase IIa study follows-up on our earlier results. In a randomized, double-blind, placebo-controlled study, we investigated the safety, efficacy and pharmacokinetics of EA-230 during experimental human endotoxaemia, a standardized controlled model of systemic inflammation induced by intravenous administration of *Escherichia coli*-derived lipopolysaccharide (LPS) to healthy volunteers.

### What is already known about this subject

- EA-230 is a β-hCG-derived immunomodulatory tetrapeptide, developed for the treatment of systemic inflammation-related disorders.
- EA-230 was shown to reduce organ dysfunction and mortality in various animal studies of systemic inflammation.
- Phase I studies showed an excellent safety profile of EA-230 and pharmacokinetics analysis revealed a very short half-life and a large volume of distribution.

### What this study adds

- No safety issues emerged in this phase IIa study in 36 volunteers.
- EA-230 attenuates the systemic inflammatory response in humans *in vivo*.
- Results of this study provide the basis for a patient study with EA-230.

## 2 | MATERIALS AND METHODS

### 2.1 | Subjects, ethics and study design

Following written informed consent, 36 healthy adult males were enrolled in this single-centre, double-blind, randomized, placebo-controlled phase IIa study. The study protocol was approved by the local ethics committee (CMO Arnhem-Nijmegen, NL56102.091.15; 2015–2231) and was prospectively registered at clinicaltrial.gov (NCT02629874). All study procedures complied with the Declaration of Helsinki, including current revisions, and Good Clinical Practice standards. Quality assurance, full data validation and monitoring of all source documents and study procedures were performed by a contract research organization (QPS, Groningen, the Netherlands).

Health status of the participants was determined by medical history, physical examination, electrocardiogram (ECG) and routine laboratory blood tests. Exclusion criteria included body mass index <18 or >30 kg/m$^2$, clinically significant illness within 2 weeks before the start of the study, known hypersensitivity to any excipients of

the drug formulations used, history of spontaneous vagal collapse, significant blood loss within 90 days prior to the study, participation in any other clinical trial within 1 month prior to the study and participation in a previous trial in which LPS was administered. Prior to and during every study procedure, subjects were not allowed to use any medication, recreational drugs, nicotine, caffeine and/or alcohol. Subjects were consecutively assigned to the 15, 45 or 90 mg/kg/h group, based on the order of their appearance on the study drug administration day. Dose regimens were based on the phase I studies using the same dosages without any safety concerns reported elsewhere in this issue.[32] Each dosage group consisted of 12 subjects; within each dosage group subjects were randomly assigned to receive either EA-230 or placebo ($n$ = 8 active study drug, $n$ = 4 placebo) making use of a randomization list. All subjects underwent endotoxaemia in combination with EA-230 or placebo, as further detailed below.

The primary endpoint was safety and tolerability, assessed by the incidence of treatment related (serious) adverse events ([S]AEs). Secondary endpoints included effects on the LPS-induced inflammatory response and pharmacokinetics.

## 2.2 | Study medication

EA-230 and placebo were supplied as solution for injection in identical 5 mL sterile single-use vials. EA-230 vials contained 300 mg/mL active substrate and placebo vials contained an osmolar equivalent of 29 mg/mL sodium chloride solution. Vials were manufactured by Halix BV (Leiden, Netherlands), quality controlled by PROXY Laboratories BV (Leiden, Netherlands), vials with EA-230 were stored at 2–8°C and placebo vials at room temperature. Before administration, the required dosage of EA-230 or placebo was diluted in 0.9% NaCl to a volume of 500 mL. Manufacturing, packaging, quality control and preparation were described in an Investigational Medicinal Product Dossier and complied with Good Manufacturing Practice requirements.

## 2.3 | Study procedures and experimental human endotoxaemia

A schematic overview of the study procedures is depicted in Figure 1.

Study subjects were administered to a fully equipped research unit at the intensive care unit of the Radboud university medical centre. The nondominant arm was cannulated for intravenous (i.v.) fluid administration and a 20-gauge arterial catheter was used for continuous blood pressure monitoring and blood sampling. As part of our standard endotoxaemia protocol,[33-35] subjects received a 1-hour infusion of 1.5 L prehydration solution (2.5% glucose/4.5% NaCl) starting at $t$ = −1 h. At $t$ = 0 h, LPS was administered, the intravenous cannula was flushed with 0.9% NaCl, and study drug treatment was initiated. Purified LPS (US Standard Reference Endotoxin *Escherichia coli* O:113) obtained from the Pharmaceutical Development Section of the National Institutes of Health (Bethesda, MD, USA) was administered as an i.v. bolus injection at a dose of 2 ng/kg body weight. Study drug treatment was administered as a 2-hour continuous i.v. infusion at 250 mL/h. Fluid administration continued at $t$ = 2 h, after cessation of study drug treatment, at 150 mL/h. During the day frequent blood samples were collected and adverse events, vital signs and endotoxaemia-related symptoms were monitored until discharge at $t$ = 8 h. Subjects returned at 24 hours, 48 hours, 7 days and 14 days after study drug administration for follow-up.

## 2.4 | Safety

Safety and tolerability assessments were performed continuously from the start of study drug treatment until 8 hours afterwards and at 4 consecutive study visits during the 14-day follow-up period. Safety parameters included vital signs (blood pressure and heart rate), 12-lead ECG and routine laboratory haematology and biochemistry. AEs were recorded throughout the complete study period. All AEs were judged by the investigator with regard to severity (mild, moderate or severe) according to Common Terminology Criteria for Adverse Events guidelines 4.0,[36] and their relation to the study drug (definitely

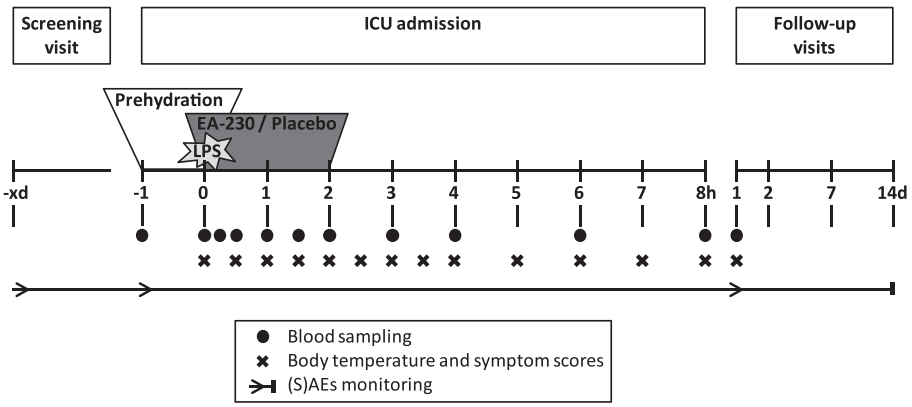


**FIGURE 1** Schematic overview of study procedures. Time points on the horizontal axes are in hours relative to LPS administration unless specified otherwise. ICU: intensive care unit; LPS: lipopolysaccharide; (S)AEs: (serious) adverse events; h: hours; d: days

related, probably related, possibly related, unlikely to be related or unrelated). LPS-induced flu-like symptoms were scored separately, as explained below, and for practical considerations excluded from safety analyses. SAEs included death, life-threatening, persistent and/or significant disability and/or incapacity and hospitalization and/or prolongation of inpatient hospitalization. Safety parameters were reported to and reviewed by an independent Data Safety Monitoring Board (DSMB) after completion of each dosing group.

## 2.5 | Plasma inflammatory mediators

Ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood samples for measurement of inflammatory parameters were obtained at time points $t$ = 0, 0.5, 1, 1.5, 2, 3, 4, 6 and 8 hours (Figure 1). Samples were immediately centrifuged at 2000 g for 10 minutes at 4°C after which plasma was stored at −80°C until analysis. Plasma levels of a core set of inflammatory mediators (interleukin [IL]-6, IL-10 and tumour necrosis factor [TNF]-α), were determined using a validated, ISO9001 certified multiplex immunoassay technology (Luminex, Austin, TX, USA) at the Laboratory of Translational Immunology of the University Medical Center Utrecht as described elsewhere.[37]

Plasma levels of IL-8, monocyte chemoattractant protein (MCP)-1, IL-1 receptor antagonist (RA), macrophage inflammatory protein (MIP)-1α and MIP-1β were determined using a Luminex assay according to the manufacturer's instructions (Milliplex; Merck Millipore, Billerica, MA, USA). Plasma levels of endothelial adhesion proteins intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion protein (VCAM)-1 were also analysed using a Luminex assay (Bio-plex; Bio-Rad, Hercules, CA, USA) according to manufacturer's instructions.

## 2.6 | Leucocyte counts and differentiation

EDTA anticoagulated blood samples were obtained at time points $t$ = −1, 0, 1, 2, 4, 8, 24 and 48 hours (Figure 1). Direct measurements of leucocyte counts and differentiations were performed using clinical routine analysis methods at the Laboratory of Clinical Chemistry of the Radboud university medical center (Sysmex XE-5000, Sysmex Nederland B.V., Etten-Leur, the Netherlands).

## 2.7 | Vital signs and endotoxaemia-induced symptoms

Heart rate, measured with a 3-lead ECG, and blood pressure were recorded every 30 seconds from a Philips MP50 patient monitor using an in-house developed system. Every 30 minutes, temperature was measured using an infrared tympanic thermometer (First-Temp Genius 2; Covidien, Dublin, Ireland), and LPS-induced flu-like symptoms (headache, nausea, shivering, muscle and back pain) were scored per symptom (0 = no symptoms, 5 = worst ever experienced, vomiting: additional 3 points), resulting in a total symptom score of 0–28.

## 2.8 | Pharmacokinetics

Blood samples for measurement of EA-230 concentrations were obtained at time points $t$ = 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 24 hours (Figure 1). Protease Inhibitor Cocktail (300 μL, P8340; Sigma-Aldrich Chemie, Zwijndrecht, The Netherlands) was added immediately after withdrawal of 3 mL EDTA-anticoagulated blood to prevent proteolytic degradation of EA-230. Hereafter, blood samples were centrifuged at 2000 $g$ for 15 minutes at 4°C and plasma samples were stored at −80°C until analysis. EA-230 concentrations were determined by a validated liquid chromatography tandem mass spectrometry assay as described elsewhere in this issue.[32] The detection range of the method was 0.5–100 ng/mL with low, medium and high quality control concentrations of 1.5, 10 and 75 ng/mL.

Pharmacokinetic analysis was performed with non-compartmental methods using WinNonLin/Phoenix version 6.3 (Pharsight Corporation, USA). The highest observed plasma concentration was defined as $C_{max}$. The area under the plasma *vs* concentration time curve from $t$ = 0 to the time of the last measured concentration ($AUC_{0\text{-last}}$) was calculated using the linear-log trapezoidal rule, with extrapolation to infinity (using $C_{last}/\beta$) to obtain the AUC from t = 0 to infinity ($AUC_{0\text{-inf}}$). The log-linear period (log concentration *vs* time) was defined by visual inspection of data points. The absolute value of the slope (β/2.303) was calculated by least squares linear regression analysis, where β is the first-order elimination rate constant. Elimination half-life ($t_{1/2}$) was calculated by the equation 0.693/β. Clearance (Cl) was calculated by dividing dose by $AUC_{0\text{-inf}}$ and volume of distribution (Vd) by dividing Cl by β.

## 2.9 | Statistical analysis

The study was regarded as exploratory in nature and sample size was based on practical considerations, exposing a limited number of subjects while obtaining the necessary safety and efficacy data. (S)AEs are summarized by treatment group, preferred term, severity and relation to the study drug. Efficacy data are presented as mean ± standard error of the mean, demographic data as mean ± standard deviation. PK parameters are presented as geometric mean and 95% confidence interval. Data were tested for normality using the Shapiro–Wilk test and a maximum normalized residual test according to Grubb was performed on efficacy data to identify significant outliers with a $P$ < .01. Differences over time between EA-230-treated and placebo-treated subjects were compared by repeated measures 2-way ANOVA (interaction term: group*time). Baseline differences in demographic data were tested using a 1-way ANOVA. Dose proportionality of dose *vs* $AUC_{0\text{-last}}$, and $C_{max}$ was assessed using 1-way ANOVA followed by a Bonferroni posthoc test on dose-normalized, log-transformed data.

All statistical analyses were performed using GraphPad Prism version 5.03 (GraphPad Software, San Diego, CA, USA). A 2-sided $P$-value <.05 was considered statistically significant.

## 3 | RESULTS

### 3.1 | Baseline characteristics and subject disposition

All study subjects received study medication according to the protocol and there were no relevant baseline differences between groups (Table 1). One subject in the 90 mg/kg/h group was excluded from all efficacy analyses because of an unusually high cytokine response (significant outlier both within the 90 mg/kg/h group and in all groups combined for IL-6 and TNF-α [Grubb's test $P < .01$]) in combination with gastrointestinal complaints that were assessed as unlikely to be related to the study drug or endotoxin administration.

### 3.2 | Safety

A summary of all (S)AEs with severity and relation to the study treatment are listed in Table 2A and their preferred terms are organized per system organ class in Table 2B.

No SAEs were reported and infusion of EA-230 during experimental endotoxaemia was well-tolerated by all subjects across dosage groups without any safety concerns and/or discontinuation of study drug administration. Accordingly, no concerns were raised by the DSMB after completion of each dosing group.

Nineteen AEs were reported by 14 subjects, all of which were mild, transient and considered not, or unlikely to be, related to study drug treatment. Seven subjects (29%) treated with EA-230 and seven placebo-treated subjects (58%) reported ≥1 AE. Of all AEs, 9 were reported in the placebo group *vs* 10 in EA-230 treated subjects, 5 of which in the highest dosing group.

Other than the expected LPS-induced alterations in vital signs and leucocyte counts discussed below, alterations in laboratory parameters, vital signs and 12-leads ECG were considered not clinically significant.

### 3.3 | Effects of EA-230 on circulating levels of inflammatory mediators and adhesion molecules during endotoxaemia

The LPS-induced increase in plasma levels of inflammatory cytokines IL-6 and IL-1RA was significantly attenuated in subjects treated with

**TABLE 1** Demographic characteristics

| | Placebo (n = 12) | 15 mg/kg/h EA-230 (*n* = 8) | 45 mg/kg/h EA-230 (n = 8) | 90 mg/kg/h EA-230 (n = 8) | *P*-value |
|---|---|---|---|---|---|
| Age, y | 23 ± 3 | 22 ± 1 | 22 ± 3 | 22 ± 2 | .62 |
| BMI, kg/m$^2$ | 23.2 ± 2.5 | 23.0 ± 1.7 | 22.7 ± 2.9 | 23.1 ± 2.7 | .98 |
| Weight, kg | 77 ± 8 | 78 ± 7 | 72 ± 9 | 79 ± 16 | .53 |
| Height, cm | 183 ± 5 | 184 ± 4 | 178 ± 8 | 184 ± 11 | .27 |
| HR, beats/min | 64 ± 9 | 65 ± 9 | 62 ± 7 | 67 ± 8 | .69 |
| MAP, mmHG | 95 ± 11 | 94 ± 3 | 92 ± 4 | 91 ± 6 | .71 |

Parameters were determined during screening visit. *BMI:* body mass index; *HR*: heart rate; *MAP*: mean arterial pressure. Data are presented as mean ± standard deviation. *P*-values were calculated using 1-way ANOVA.

**TABLE 2A** Summary of adverse events

| | Placebo (*n* = 12) *n* (%) | 15 mg/kg/h EA-230 (*n* = 8) *n* (%) | 45 mg/kg/h EA-230 (*n* = 8) *n* (%) | 90 mg/kg/h EA-230 (*n* = 8) *n* (%) | Overall (*n* = 36) *n* (%) |
|---|---|---|---|---|---|
| Any AE | 7 (58.3) | 2 (25.0) | 2 (25.0) | 3 (37.5) | 14 (38.9) |
| Any serious AE | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Discontinued due to AE | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Concomitant medication given | 0 (0.0) | 0 (0.0) | 1 (12.5) | 0 (0.0) | 1 (2.8) |
| AE of mild intensity | 7 (58.3) | 2 (25.0) | 2 (25.0) | 3 (37.5) | 14 (38.9) |
| AE of moderate intensity | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| AE of severe intensity | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Definitely related AE | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Probably related AE | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Possibly related AE | 1 (8.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (2.8) |
| Unlikely related/unrelated AE | 6 (50.0) | 2 (25.0) | 2 (25.0) | 3 (37.5) | 13 (36.1) |

*AE*, adverse events; *n*, number of subjects.

**TABLE 2B** Summary of adverse events by system organ class and preferred term

| Dosage groups<br>System organ class and preferred term | Placebo<br>(*n* = 12)<br>*n* (%) e | 15 mg/kg/h EA-230<br>(*n* = 8)<br>*n* (%) e | 45 mg/kg/h EA-230<br>(*n* = 8)<br>*n* (%) e | 90 mg/kg/h EA-230<br>(*n* = 8)<br>*n* (%) e | Overall<br>(*n* = 36)<br>*n* (%) e |
|---|---|---|---|---|---|
| Number of subjects with at least 1 adverse event | 7 (58.3) 9 | 2 (25.0) 3 | 2 (25.0) 2 | 3 (37.5) 5 | 14 (38.9) 19 |
| General disorders | 4 (33.3) 5 | 1 (12.5) 1 | 0 (0.0) 0 | 1 (12.5) 1 | 6 (16.7) 7 |
| - Infusion site reaction | 4 (33.3) 4 | 1 (12.5) 1 | 0 (0.0) 0 | 1 (12.5) 1 | 6 (16.7) 6 |
| - Feeling hot | 1 (8.3) 1 | 0 (0.0) 0 | 0 (0.0) 0 | 0 (0.0) 0 | 1 (2.8) 1 |
| Nervous system disorders | 1 (8.3) 1 | 1 (12.5) 1 | 0 (0.0) 0 | 0 (0.0) 0 | 2 (5.6) 2 |
| - Head discomfort | 1 (8.3) 1 | 1 (12.5) 1 | 0 (0.0) 0 | 0 (0.0) 0 | 2 (5.6) 2 |
| Gastrointestinal disorders | 0 (0.0) 0 | 1 (12.5) 1 | 0 (0.0) 0 | 1 (12.5) 2 | 2 (5.6) 3 |
| - Nausea | 0 (0.0) 0 | 0 (0.0) 0 | 0 (0.0) 0 | 1 (12.5) 1 | 1 (2.8) 1 |
| - Upper abdominal pain | 0 (0.0) 0 | 0 (0.0) 0 | 0 (0.0) 0 | 1 (12.5) 1 | 1 (2.8) 1 |
| - Soft faeces | 0 (0.0) 0 | 1 (12.5) 1 | 0 (0.0) 0 | 0 (0.0) 0 | 1 (2.8) 1 |
| Musculoskeletal disorders | 1 (8.3) 1 | 0 (0.0) 0 | 2 (25.0) 2 | 2 (25.0) 2 | 5 (13.9) 5 |
| - Pain in extremity | 0 (0.0) 0 | 0 (0.0) 0 | 1 (12.5) 1 | 2 (25.0) 2 | 3 (8.3) 3 |
| - Back pain | 0 (0.0) 0 | 0 (0.0) 0 | 1 (12.5) 1 | 0 (0.0) 0 | 1 (2.8) 1 |
| - Muscular weakness | 1 (8.3) 1 | 0 (0.0) 0 | 0 (0.0) 0 | 0 (0.0) 0 | 1 (2.8) 1 |
| Respiratory disorders | 1 (8.3) 1 | 0 (0.0) 0 | 0 (0.0) 0 | 0 (0.0) 0 | 1 (2.8) 1 |
| - Rhinorrhoea | 1 (8.3) 1 | 0 (0.0) 0 | 0 (0.0) 0 | 0 (0.0) 0 | 1 (2.8) 1 |
| Infectious disorders | 1 (8.3) 1 | 0 (0.0) 0 | 0 (0.0) 0 | 0 (0.0) 0 | 1 (2.8) 1 |
| - Tonsillitis | 1 (8.3) 1 | 0 (0.0) 0 | 0 (0.0) 0 | 0 (0.0) 0 | 1 (2.8) 1 |

*AE*, adverse events; e, number of events; *n*, number of subjects.

90 mg/kg/h EA-230 compared to the placebo group (% reduction in AUC of 48 and 33 respectively), but not of TNF-α and IL-10 (% increment in AUC of 1 and 33 respectively; Figure 2). The other dosages of EA-230 had no effects on either of these cytokines, these data are depicted in Supplemental data file 1. Treatment with the highest dose of EA-230 also significantly attenuated circulating levels of chemokines IL-8, MCP-1, MIP1-α and MIP1-β (% reduction in AUC of 28, 28, 14 and 16 respectively; Figure 3), and plasma concentrations of the endothelial adhesion molecule VCAM-1, but not intercellular adhesion molecule-1 (% reduction in AUC of 19 and 5 respectively; Figure 4). Again, the lower dosages of EA-230 had no effects on any of these mediators (Supplemental data file 1).

## 3.4 | Effects of EA-230 on leucocyte counts and differentiation during endotoxaemia

LPS administration typically caused initial leucopenia during the 1st hour followed by a profound increase in numbers of circulating leucocytes (Figure 5A). Treatment with 90 mg/kg/h EA-230 did not affect the initial decrease in leucocyte counts, whereas it subsequently resulted in higher leucocyte counts compared to the placebo group, an effect mainly attributed to increased numbers of circulating neutrophils and lymphocytes, but not monocytes (Figures 5). The lower dosages of EA-230 did not influence leucocyte numbers or differentiation compared to the LPS-response in the placebo group (Supplemental data file 1).

## 3.5 | Effects of EA-230 on vital signs and symptoms during endotoxaemia

Endotoxaemia resulted in fever (approximately an increase in body temperature of 2°C; Figure 6A), development of influenza-like symptoms (Figure 6B), a decrease in mean arterial pressure (approximately 10 mmHg; Figure 6C), and an increase in heart rate (approximately 30 beats/min; Figure 6D). Fever peaked at 3–3.5 hours following LPS administration and was significantly lower in subjects treated with 90 mg/kg/h EA-230 compared to placebo (increase of 1.3 ± 0.2°C vs. 1.8 ± 0.1°C, respectively, at $t$ = 3.5; Figure 6A). Flu-like symptoms peaked at 1.5 hours after LPS administration and were approximately halved in subjects treated with 90 mg/kg/h EA-230 compared to placebo (4.0 ± 1.2 points vs. 7.4 ± 1.0 points respectively; Figure 6B). The LPS-induced effects on mean arterial pressure and heart rate were not altered by EA-230 in any of the dosages (Supplemental data file 1).

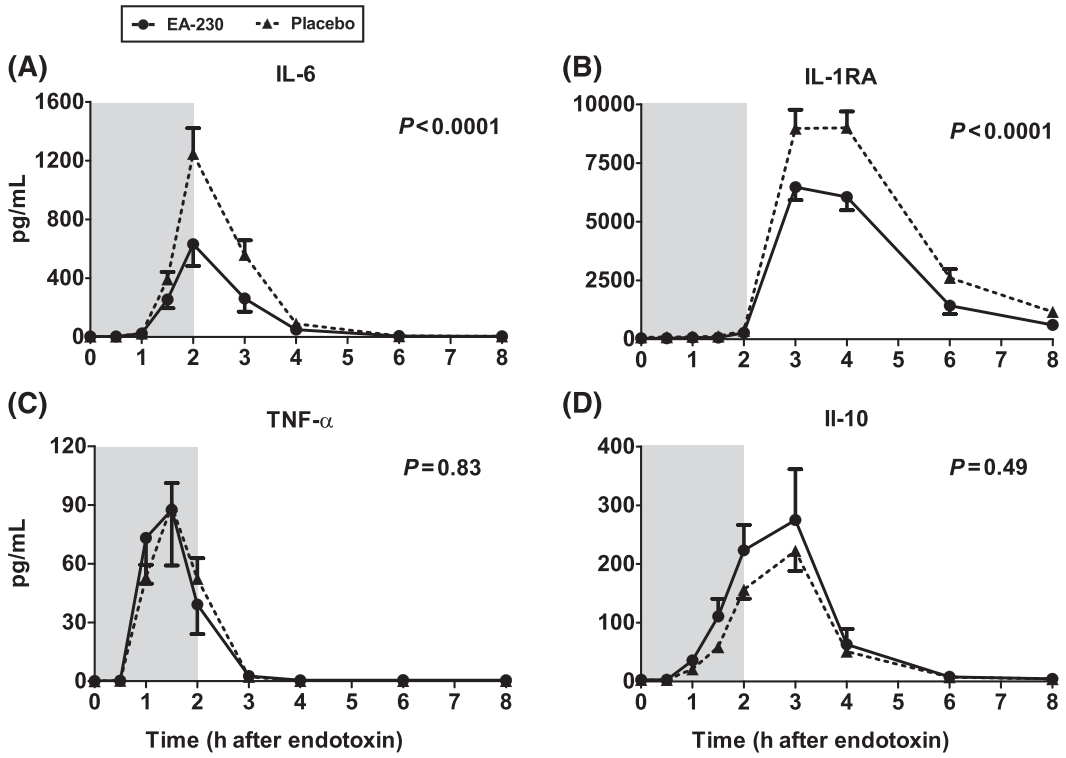


**FIGURE 2** Plasma levels of cytokines during endotoxaemia. A, Interleukin (IL)-6, B, IL-1 receptor antagonist (RA), C, tumour necrosis factor (TNF)-α, D, IL-10. Data are represented as means with standard error of the mean of $n$ = 7 in the EA-230 90 mg/kg/h group and $n$ = 12 in the placebo group. Grey box indicates the period in which the active group received EA-230. $P$-values between groups were calculated using repeated measures 2-way analysis of variance (ANOVA, interaction term)

### 3.6 | Pharmacokinetics of EA-230 during endotoxaemia

Plasma EA-230 concentration–time profiles and dose linearity are illustrated in Figure 7. PK parameters are summarized in Table 3. Figure 7A illustrates that stable plasma concentrations were reached for all dosage groups within 15 minutes after start of study treatment and during the 2-hour continuous administration period, followed by a very rapid decline in plasma concentrations after cessation of study treatment. Due to this very swift decline, the elimination rate constant β (and $t_{1/2}$, Cl and Vd) could only be calculated in a limited number of subjects for the first 2 dosage groups (15 and 45 mg/kg/h). These analyses revealed rapid plasma elimination, with a $t_{1/2}$ of <0.77 h in the 15 mg/k/h group ($n$ = 3) and <0.16 h in the 45 mg/kg/h ($n$ = 5). In the highest dosing group, β could be estimated for all 8 subjects, and a large volume of distribution (range: 1.3–3.8 L/kg), a short elimination $t_{1/2}$ (range: 0.12–0.24 h) and a corresponding rapid clearance rate (range: 7–12 L/h/kg) for EA-230 was apparent. The dosage increase from 15 mg/kg/h to 90 mg/kg/h resulted in proportional increases in $C_{max}$ and $AUC_{0\text{-last}}$ (Figure 7B–C, Table 3).

## 4 | DISCUSSION

In this double-blind, placebo-controlled phase IIa study, we studied the safety and immunomodulatory effects of the β-HCG derived peptide EA-230 in healthy volunteers during experimental endotoxaemia. Our data demonstrate that EA-230 profoundly attenuates the systemic inflammatory response in humans *in vivo*. Furthermore, we show that EA-230 is well-tolerated and seems to be safe for administration under inflammatory conditions.

A 2-hour infusion of EA-230 in the highest tested dosage of 90 mg/kg/h dampened the endotoxin-induced proinflammatory innate immune response, exemplified by attenuated plasma levels of the pro-inflammatory cytokine IL-6, chemokines IL-8, MCP-1, MIP-1a and MIP-1b, and endothelial adhesion molecule VCAM-1. Levels of the archetypal anti-inflammatory cytokine IL-10 were not affected by the study drug. In addition to these biochemical findings, modulation of the inflammatory response by EA-230 was supported clinically by a significant reduction in endotoxin-induced fever and influenza-like symptoms. These findings are in line with preclinical studies that demonstrated that EA-230 attenuates cytokine production, prevents tissue influx of neutrophils[24,26,28] and ameliorates inflammation-related clinical symptoms[24-26,28] in various models of systemic inflammation. Our data also reveal that, following the expected LPS-induced initial leucopenia (predominantly mediated by endothelial sequestration of lymphocytes and monocytes), the subsequent increase in numbers of circulating leucocytes (predominantly due to increased neutrophil numbers released from the bone marrow) was more pronounced in EA-230-treated subjects.

The substantial attenuation of the endotoxaemia-induced IL-6 response in EA-230-treated subjects may be of clinical relevance, as

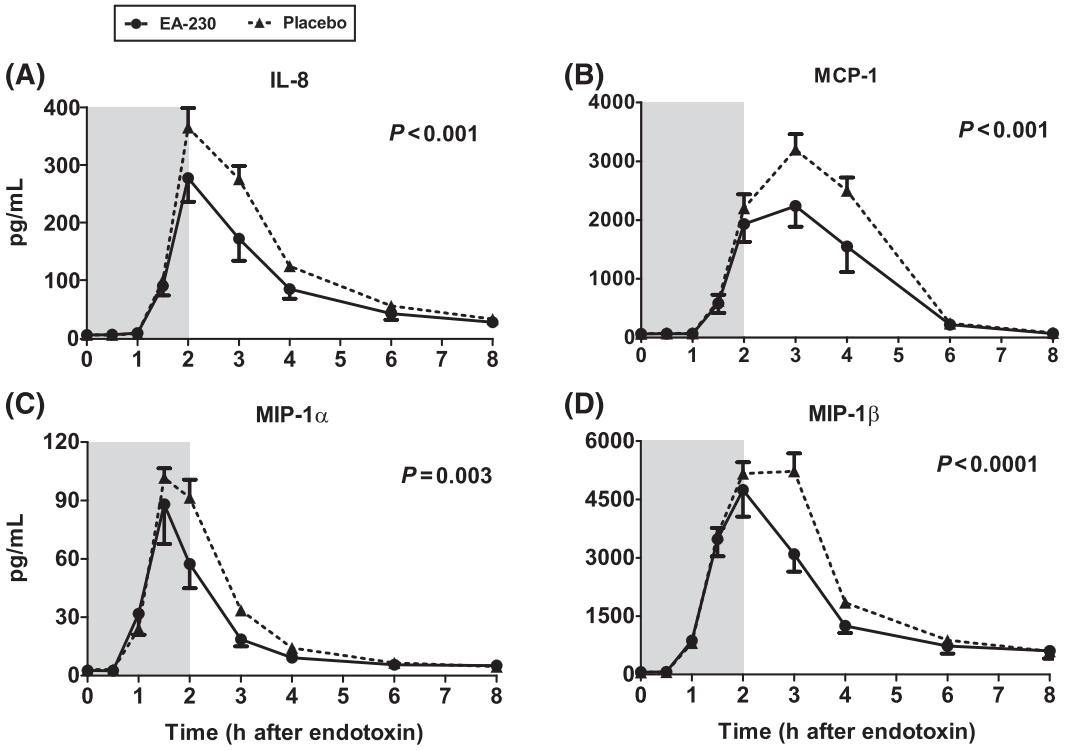


**FIGURE 3** Plasma levels of chemokines during endotoxaemia. A, Interleukin (IL)-8, B, monocyte chemoattractant protein (MCP)-1, C, macrophage inflammatory protein (MIP)-1α D, MIP-1β. Data are represented as means with standard error of the mean of *n* = 7 in the EA-230 90 mg/kg/h group and *n* = 12 in the placebo group. Grey box indicates the period in which the active group received EA-230. *P*-values between groups were calculated using repeated measures 2-way analysis of variance (ANOVA, interaction term)

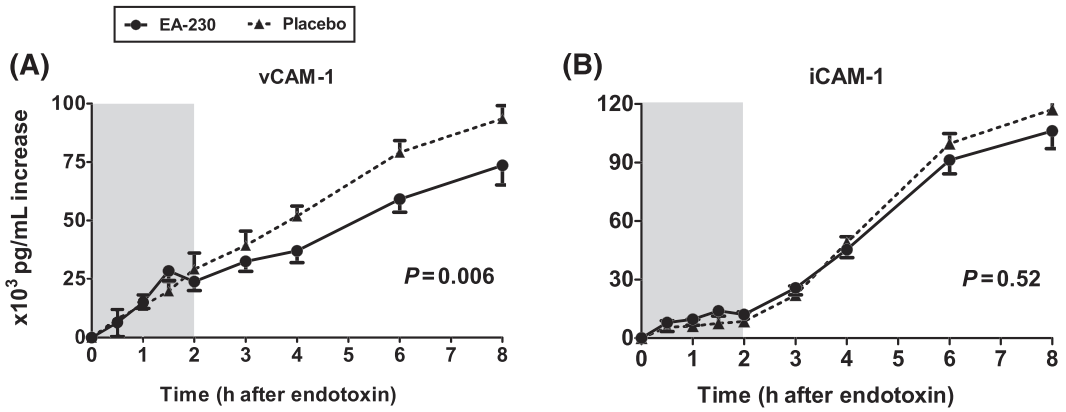


**FIGURE 4** Plasma levels of endothelial cell adhesion molecules during endotoxaemia. A, Vascular cell adhesion molecule (vCAM)-1; B, intercellular adhesion molecule (iCAM)-1. Data are represented as means with standard error of the mean of *n* = 7 in the EA-230 90 mg/kg/h group and *n* = 12 in the placebo group. Grey box indicates the period in which the active group received EA-230. *P*-values between groups were calculated using repeated measures 2-way analysis of variance (ANOVA, interaction term)

IL-6 is implicated to play a central role in various inflammatory disorders. As such, this cytokine is regarded to be a reliable marker reflecting the magnitude of systemic inflammation, e.g. following elective surgery[38] or in patients with pneumonia.[39] Furthermore, IL-6 levels are associated with organ failure and impaired (functional) outcome following severe injury,[40] cardiac surgery,[41] cardiac arrest,[42] stroke[43] and sepsis.[44] With respect to renal function, IL-6 plays a critical role in the development of AKI,[45] independently predicting mortality in patients starting dialysis[46] and predicting AKI following cardiac surgery.[41] In this perspective, our results are promising and EA-230 may show efficacy on these clinical endpoints in future studies.

Administration of EA-230 was well-tolerated throughout the conduct of the trial and no safety issues were raised during frequent independent interim safety analyses by the DSMB and in the final results. No dose-dependency for adverse events was observed and

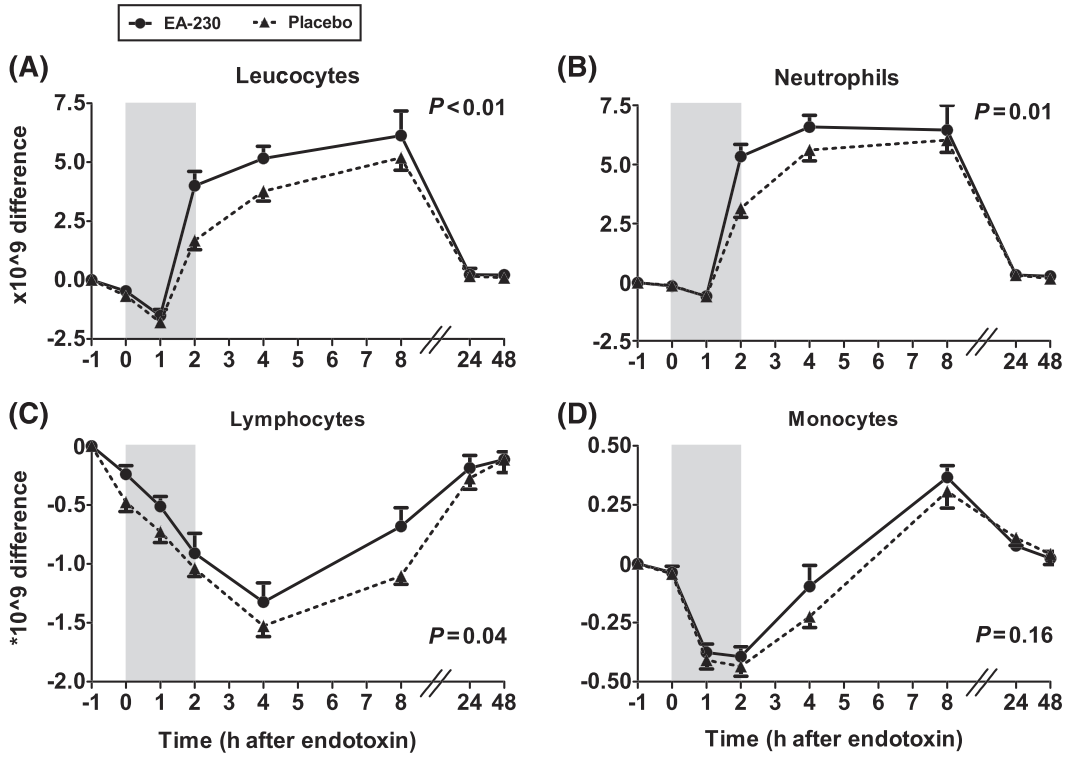


**FIGURE 5** Circulating leucocyte numbers during endotoxaemia. A, Leucocytes, B, neutrophils, C, lymphocytes, D, monocytes. Data are represented as means with standard error of the mean of $n$ = 7 in the EA-230 90 mg/kg/h group and $n$ = 12 in the placebo group. Grey box indicates the period in which the active group received EA-230. *P*-values between groups were calculated using repeated measures 2-way analysis of variance (ANOVA, interaction term)

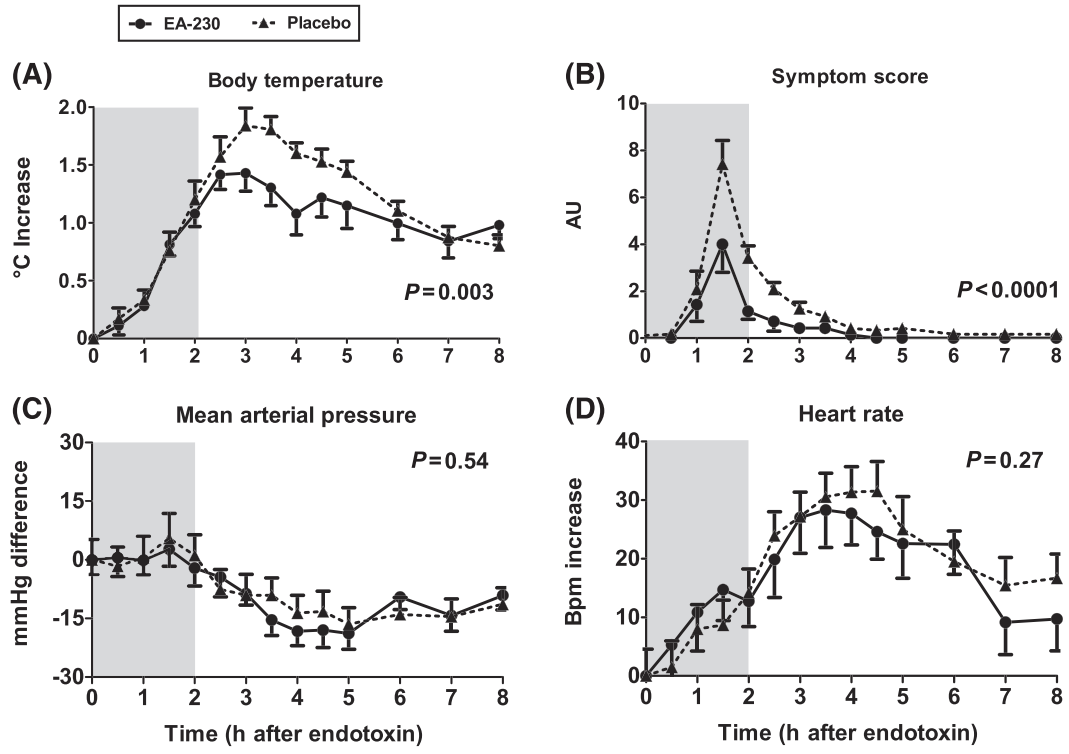


**FIGURE 6** Clinical variables during endotoxaemia. A, Increase in body temperature B, symptom scores, C, mean arterial pressure, D, heart rate. Data are represented as means with standard error of the mean of $n$ = 7 in the EA-230 90 mg/kg/h group and $n$ = 12 in the placebo group. Grey box indicates the period in which the active group received EA-230. *P*-values between groups were calculated using repeated measures 2-way analysis of variance (ANOVA, interaction term). AU: arbitrary unit; Bpm: beats/min

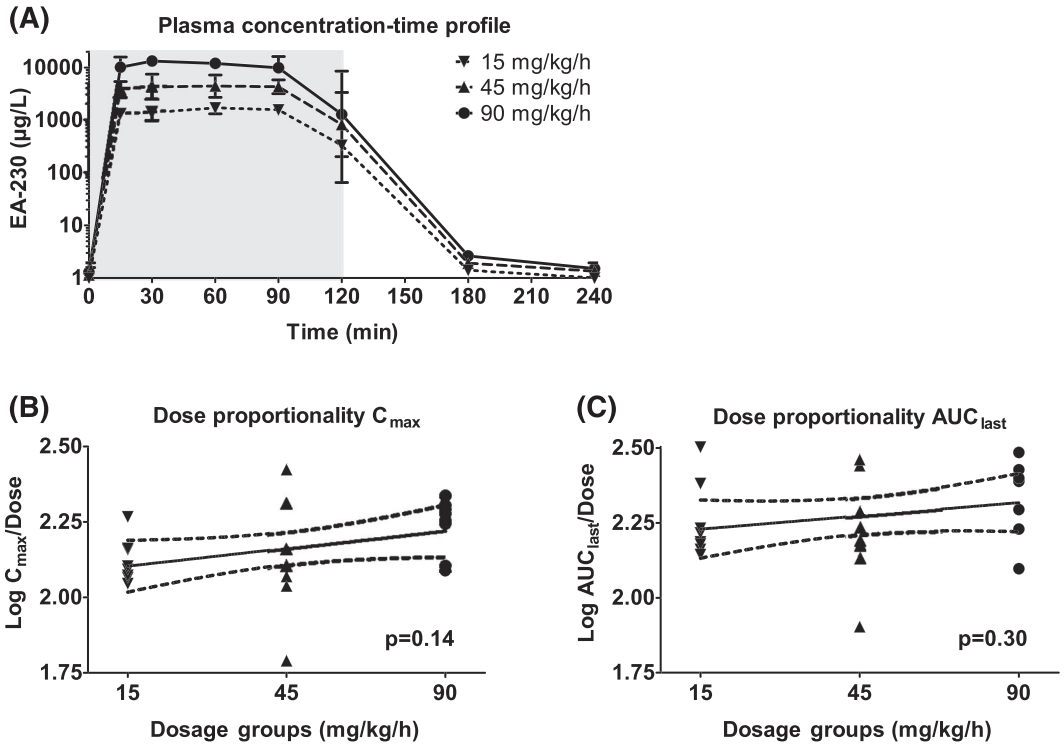


**FIGURE 7** Pharmacokinetics of EA-230. A, Plasma concentration-time profiles of EA-230. The grey area indicates the study drug administration period. B, C, Dose proportionality of dose-normalized, log-transformed exposure parameters $C_{max}$ (B), and $AUC_{0\text{-last}}$ (C). Linear regression lines are shown, dotted lines indicate the 95% confidence interval, a *P*-value of <.05 would have indicated nonproportionality. Data are expressed as geometric means and 95% confidence interval

**TABLE 3** Pharmacokinetic parameters of EA-230

| Pharmacokinetic parameters | *n* | 15 mg/kg/h EA-230 | *n* | 45 mg/kg/h EA-230 | *n* | 90 mg/kg/h EA-230 |
|---|---|---|---|---|---|---|
| $AUC_{0\text{-last}}$ (h µg/L) | 8 | 2672 (2097–3403) | 8 | 7647 (5431–10766) | 8 | 19658 (15428–25046) |
| $AUC_{0\text{-inf}}$ (h µg/L) | 3 | 3349 (1244–9014) | 5 | 6519 (4243–10015) | 8 | 19658 (15429–25046) |
| $C_{max}$ (µg/L) | 8 | 1983 (1725–2279) | 8 | 6030 (4190–8676) | 8 | 15657 (13100–18714) |
| $T_{1/2}$ (h) | 3 | 0.15 (0.03–0.77) | 5 | 0.11 (0.08–0.16) | 8 | 0.17 (0.12–0.24) |
| CL (L/h/kg) | 3 | 9 (3–24) | 5 | 14 (9–21) | 8 | 9 (7–12) |
| Vd (L/kg) | 3 | 1.9 (0.1–2.6) | 5 | 2.3 (1.3–3.9) | 8 | 2.2 (1.3–3.8) |

Data expressed as geometric means and 95% CI. $T_{1/2}$: elimination half-life; $C_{max}$: highest observed plasma concentration; $AUC_{0\text{-last}}$: the area under the plasma *vs* concentration time curve from t = 0 to the time of the last measured concentration; $AUC_{0\text{-inf}}$: the area under the plasma *vs* concentration time curve from *t* = 0 to infinity extrapolated; *Cl*: plasma clearance; *Vd*: volume of distribution.

the percentage of subjects with 1 or more AE tended to be less common in the highest dosing group compared to the placebo group (respectively 58 *vs* 38%). Furthermore, all AEs in the active groups were of mild intensity and regarded unlikely or unrelated to study drug treatment. These results are consistent with earlier human phase I trials focusing on safety of EA-230 in the absence of systemic inflammation reported elsewhere in this issue[32] and indicate that EA-230 seems to be safe in dosages up to 90 mg/kg/h. Further safety data with higher dosages and/or longer administration duration would be needed to study a possible exposure-dependent effect.

One subject was found to be a statistically significant outlier (per Grubb's test) and was excluded for the primary efficacy analyses. When this volunteer was not excluded, the effects of EA-230 on clinical endpoints remained statistically significant, while the effects on some of the cytokines went from statistically significant to a trend.

Data on the pharmacokinetic profile of EA-230 reveal a large volume of distribution, a very rapid systemic clearance and accordingly a short half-life. A finding of interest is the substantially higher plasma concentrations of EA-230 compared to the phase I continuous dosing study reported elsewhere in this issue,[32] which was performed and analysed within the same institute, using exactly the same dosing regimens (15, 45 and 90 mg/kg/h for 2-hours). The elimination rates (β) were, however, comparable to those found in the phase I continuous dosage study. The observed increase in plasma concentrations are therefore accompanied

by a decrease in Vd and Cl. These findings indicate that LPS-induced systemic inflammation affected the pharmacokinetic behaviour of EA-230 by decreasing the volume of distribution and increasing plasma concentration of EA-230. As the exact mode of action of EA-230 still remain to be elucidated, it is unclear by what mechanism(s) the inflammatory response affects the PK of EA-230. Receptor competition with an inflammation-dependent ligand or decreased internalization of EA-230 during systemic inflammation may be possible explanations for the limitation of the drug's volume of distribution and subsequent increase in plasma concentrations. Taken together, the PK-profile indicates that EA-230 distributes over a large volume and is very rapidly metabolized with plasma clearance exceeding both renal and portal flow. This profile is affected by systemic inflammation, as reflected by the observed potentiated plasma concentrations and a lower volume of distribution during experimental human endotoxaemia.

Several limitations of this study need to be addressed. The experimental human endotoxaemia model elicits a predictable and reproducible systemic inflammatory response and therefore provides a suitable model for a proof-of-principle study evaluating an immunomodulatory compound. However, as with any model, it has several limitations. First, LPS is a specific toll-like receptor-4 ligand and is administrated in a single bolus. As such, it recapitulates a 1-hit immunological insult activating a specific pathway, which may differ from the immune response observed in clinical practice, which is often more sustained and caused by various pathogens or other immunological insults activating multiple pathways. Second, young healthy male subjects were included to minimize statistical dispersion, as age, female sex and comorbidities are known factors accounting for heterogeneity in inflammatory responses.[47,48] This markedly differs from clinical practice, where diseases associated with a systemic inflammatory response occur in people of all ages, both sexes, and are frequently complicated by 1 or more comorbidities as well as the use of comedication. Therefore, the generalizability of our results to the patient population remains to be determined. Third, although all PK parameters could be determined in all subjects of the highest dosing group, the pharmacokinetic characteristics of EA-230 (e.g. its very short half-life) precluded assessment of elimination constant-dependent parameters in several subjects of the lower dosage groups. However, in these subjects, plasma concentrations approaching zero (after approximately 5 times $T_{1/2}$) were reached within an hour, suggesting a $T_{1/2}$, of <0.2 h, which is in line with the data obtained in subjects in whom elimination constant-dependent parameters could be assessed. Finally, the exact mechanism(s) by which EA-230 modulates the immune response remain unclear and may have consequences for the optimal timing of administration. In the current study, steady-state concentrations EA-230 were present during the orchestration phase and peak (e.g. IL-6) of the inflammatory response. In clinical practice, the timing of the inflammatory insult(s) is/are generally unknown and a subsequent more diffuse and longer lasting inflammatory response is often observed. Therefore, no definite conclusions regarding the optimal timing of administration of EA-230 for future clinical research can be drawn from this study.

In conclusion, the present proof-of-principle study demonstrates that EA-230 seems to be safe and attenuates the systemic inflammatory response in humans. Substantially higher plasma concentrations of EA-230 were observed in subjects with systemic inflammation compared to non-inflamed subjects, which was explained by a decrease in volume of distribution and clearance, but not by differences in the elimination constant. These inflammation-related effects on pharmacokinetic characteristics of EA-230 need to be further elucidated. The favourable safety profile of EA-230 and promising immunomodulatory results pave the way for a phase IIb clinical trial to assess the anti-inflammatory and tissue-protective effects of EA-230 in patients.[49]

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## COMPETING INTERESTS

P.P. received consulting fees and travel reimbursements from EBI. All other authors have no competing interests to declare.

All protocols, including amendments, were approved by the local ethics committee (CMO Arnhem-Nijmegen, NL56102.091.15; 2015–2231). The study was conducted in accordance with the principles of the Declaration of Helsinki, and in compliance with the International Conference on Harmonisation E6 Guideline for Good Clinical Practice (CPMP/ICH/135/95). All healthy volunteers who participated in the study provided written informed consent before the start of any study-related procedures.

## CONTRIBUTORS

All authors participated in the conception, design, and/or coordination of study components. All authors have critically reviewed and approved the final manuscript for publication

## REFERENCES

1. Balk RA. Systemic inflammatory response syndrome (SIRS): where did it come from and is it still relevant today? *Virulence*. 2014;5(1):20-26.
2. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA*. 2016;315(8):801-810.
3. Vincent JL, Sakr Y, Sprung CL, et al. Sepsis in European intensive care units: results of the SOAP study. *Crit Care Med*. 2006;34(2):344-353.
4. Dulhunty JM, Lipman J, Finfer S. Does severe non-infectious SIRS differ from severe sepsis? Results from a multi-centre Australian and New Zealand intensive care unit study. *Intensive Care Med*. 2008;34(9):1654-1661.
5. Thiele RH, Isbell JM, Rosner MH. AKI associated with cardiac surgery. *Clin J Am Soc Nephrol*. 2015;10(3):500-514.
6. Hoste EA, Bagshaw SM, Bellomo R, et al. Epidemiology of acute kidney injury in critically ill patients: the multinational AKI-EPI study. *Intensive Care Med*. 2015;41(8):1411-1423.
7. Rabb H, Griffin MD, McKay DB, et al. Inflammation in AKI: current understanding, key questions, and knowledge gaps. *J Am Soc Nephrol*. 2016;27(2):371-379.

8. Payen D, Lukaszewicz AC, Legrand M, et al. A multicentre study of acute kidney injury in severe sepsis and septic shock: association with inflammatory phenotype and HLA genotype. *PLoS One*. 2012;7(6):e35838.
9. Cohen J, Vincent JL, Adhikari NK, et al. Sepsis: a roadmap for future research. *Lancet Infect Dis*. 2015;15(5):581-614.
10. Lord JM, Midwinter MJ, Chen YF, et al. The systemic immune response to trauma: an overview of pathophysiology and treatment. *Lancet*. 2014;384(9952):1455-1465.
11. Landis RC, Brown JR, Fitzgerald D, et al. Attenuating the systemic inflammatory response to adult cardiopulmonary bypass: a critical review of the evidence base. *J Extra Corpor Technol*. 2014;46(3):197-211.
12. Munoz-Suano A, Hamilton AB, Betz AG. Gimme shelter: the immune system during pregnancy. *Immunol Rev*. 2011;241(1):20-38.
13. Racicot K, Kwon JY, Aldo P, Silasi M, Mor G. Understanding the complexity of the immune system during pregnancy. *Am J Reprod Immunol*. 2014;72(2):107-116.
14. Vukusic S, Hutchinson M, Hours M, et al. Pregnancy and multiple sclerosis (the PRIMS study): clinical predictors of post-partum relapse. *Brain*. 2004;127(Pt 6):1353-1360.
15. Kaaja RJ, Greer IA. Manifestations of chronic disease during pregnancy. *JAMA*. 2005;294(21):2751-2757.
16. Hazes JM, Coulie PG, Geenen V, et al. Rheumatoid arthritis and pregnancy: evolution of disease activity and pathophysiological considerations for drug use. *Rheumatology (Oxford)*. 2011;50(11):1955-1968.
17. Murase JE, Chan KK, Garite TJ, Cooper DM, Weinstein GD. Hormonal effect on psoriasis in pregnancy and post partum. *Arch Dermatol*. 2005;141(5):601-606.
18. Adcock EW 3rd, Teasdale T, August CS, et al. Human chorionic gonadotropin: its possible role in maternal lymphocyte suppression. *Science*. 1973;181(4102):845-847.
19. Wan H, Versnel MA, Leijten LM, et al. Chorionic gonadotropin induces dendritic cells to express a tolerogenic phenotype. *J Leukoc Biol*. 2008;83(4):894-901.
20. Bansal AS, Bora SA, Saso S, Smith JR, Johnson MR, Thum MY. Mechanism of human chorionic gonadotrophin-mediated immunomodulation in pregnancy. *Expert Rev Clin Immunol*. 2012;8(8):747-753.
21. Schumacher A, Brachwitz N, Sohr S, et al. Human chorionic gonadotropin attracts regulatory T cells into the fetal-maternal interface during early human pregnancy. *J Immunol*. 2009;182(9):5488-5497.
22. Khan NA, Benner R. Human chorionic gonadotropin: a model molecule for oligopeptide-based drug discovery. *Endocr Metab Immune Disord Drug Targets*. 2011;11(1):32-53.
23. Khan NA, Khan A, Savelkoul HF, Benner R. Inhibition of septic shock in mice by an oligopeptide from the beta-chain of human chorionic gonadotrophin hormone. *Hum Immunol*. 2002;63(1):1-7.
24. Khan NA, Susa D, van den Berg JW, et al. Amelioration of renal ischaemia-reperfusion injury by synthetic oligopeptides related to human chorionic gonadotropin. *Nephrol Dial Transplant*. 2009;24(9):2701-2708.
25. Gueler F, Shushakova N, Mengel M, et al. A novel therapy to attenuate acute kidney injury and ischemic allograft damage after allogenic kidney transplantation in mice. *PLoS One*. 2015;10(1):e0115709.
26. Khan NA, Vierboom MP, van Holten-Neelen C, et al. Mitigation of septic shock in mice and rhesus monkeys by human chorionic gonadotrophin-related oligopeptides. *Clin Exp Immunol*. 2010;160(3):466-478.
27. van der Zee M, Dik WA, Kap YS, et al. Synthetic human chorionic gonadotropin-related oligopeptides impair early innate immune responses to *Listeria monocytogenes* in mice. *J Infect Dis*. 2010;201(7):1072-1080.
28. van den Berg HR, Khan NA, van der Zee M, et al. Synthetic oligopeptides related to the [beta]-subunit of human chorionic gonadotropin attenuate inflammation and liver damage after (trauma) hemorrhagic shock and resuscitation. *Shock*. 2009;31(3):285-291.
29. van der Zee M, van den Berg JW, van Holten-Neelen C, Dik WA. The beta-human chorionic gonadotropin-related peptide LQGV exerts anti-inflammatory effects through activation of the adrenal gland and glucocorticoid receptor in C57BL/6 mice. *J Immunol*. 2010;185(9):5066-5073.
30. van den Berg JW, Dik WA, van der Zee M, et al. The beta-human chorionic gonadotropin-related peptide LQGV reduces mortality and inflammation in a murine polymicrobial sepsis model. *Crit Care Med*. 2011;39(1):126-134.
31. Zamorina SA, Shirshev SV. Oligopeptides of chorionic gonadotropin beta-subunit in induction of T cell differentiation into Treg and Th17. *Bull Exp Biol Med*. 2015;160(1):72-75.
32. van Groenendael R, Aarnoutse R, Kox M, van Eijk LT, Pickkers P. Pharmacokinetics, safety and tolerability of the novel β-hCG derived immunomodulatory compound, EA-230. *Br J Clin Pharmacol*. 2019;85(7):1572-1584.
33. Dorresteijn MJ, van Eijk LT, Netea MG, Smits P, van der Hoeven JG, Pickkers P. Iso-osmolar prehydration shifts the cytokine response towards a more anti-inflammatory balance in human endotoxemia. *J Endotoxin Res*. 2005;11(5):287-293.
34. van Eijk LT, Pickkers P, Smits P, Bouw MP, van der Hoeven JG. Severe vagal response after endotoxin administration in humans. *Intensive Care Med*. 2004;30(12):2279-2281.
35. van Lier D, Geven C, Leijte GP, Pickkers P. Experimental human endotoxemia as a model of systemic inflammation. *Biochimie*. 2019;159:99-106.
36. NIH. Common Terminology Criteria for Adverse Events (CTCAE) v4.0. 2009. http://evsncinihgov/ftp1/CTCAE/Abouthtml (May 28 (v4.03: June 14 2010)).
37. de Jager W, Prakken BJ, Bijlsma JW, Kuis W, Rijkers GT. Improved multiplex immunoassay performance in human plasma and synovial fluid following removal of interfering heterophilic antibodies. *J Immunol Methods*. 2005;300(1–2):124-135.
38. Watt DG, Horgan PG, McMillan DC. Routine clinical markers of the magnitude of the systemic inflammatory response after elective operation: a systematic review. *Surgery*. 2015;157(2):362-380.
39. Kellum JA, Kong L, Fink MP, et al. Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. *Arch Intern Med*. 2007;167(15):1655-1663.
40. Cuschieri J, Bulger E, Schaeffer V, et al. Early elevation in random plasma IL-6 after severe injury is associated with development of organ failure. *Shock*. 2010;34(4):346-351.
41. Zhang WR, Garg AX, Coca SG, et al. Plasma IL-6 and IL-10 concentrations predict AKI and long-term mortality in adults after cardiac surgery. *J Am Soc Nephrol*. 2015;26(12):3123-3132.
42. Vaahersalo J, Skrifvars MB, Pulkki K, et al. Admission interleukin-6 is associated with post resuscitation organ dysfunction and predicts long-term neurological outcome after out-of-hospital ventricular fibrillation. *Resuscitation*. 2014;85(11):1573-1579.
43. Bustamante A, Sobrino T, Giralt D, et al. Prognostic value of blood interleukin-6 in the prediction of functional outcome after stroke: a systematic review and meta-analysis. *J Neuroimmunol*. 2014;274(1–2):215-224.

44. Remick DG, Bolgos GR, Siddiqui J, Shin J, Nemzek JA. Six at six: interleukin-6 measured 6 h after the initiation of sepsis predicts mortality over 3 days. *Shock*. 2002;17(6):463-467.
45. Nechemia-Arbely Y, Barkan D, Pizov G, et al. IL-6/IL-6R axis plays a critical role in acute kidney injury. *J Am Soc Nephrol*. 2008;19(6):1106-1115.
46. Pecoits-Filho R, Barany P, Lindholm B, Heimburger O, Stenvinkel P. Interleukin-6 is an independent predictor of mortality in patients starting dialysis treatment. *Nephrol Dial Transplant*. 2002;17(9):1684-1688.
47. Howell KW, Cleveland JC Jr, Meng X, et al. Interleukin 6 production during cardiac surgery correlates with increasing age. *J Surg Res*. 2016;201(1):76-81.
48. van Eijk LT, Dorresteijn MJ, Smits P, van der Hoeven JG, Netea MG, Pickkers P. Gender differences in the innate immune response and vascular reactivity following the administration of endotoxin to human volunteers. *Crit Care Med*. 2007;35(6):1464-1469.
49. van Groenendael R, Beunders R, Hofland J, et al. The safety, tolerability, and effects on the systemic inflammatory response and renal function of the human chorionic gonadotropin hormone-derivative EA-230 following on-pump cardiac surgery (the EASI Study): protocol for a randomized, double-blind, placebo-controlled phase 2 study. *JMIR Res Protoc*. 2019;8(2):e11441.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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